

Using FlowCAM technology to measure high frequency spatial and temporal variation in phytoplankton and zooplankton species composition and develop state-of-the-art plankton monitoring programs

#0056

Technical Panel Review

Proposal Name: Using FlowCAM technology to measure high frequency spatial and temporal variation in phytoplankton and zooplankton species composition and develop state-of-the-art plankton monitoring programs

Applicant Organization: California Department of Water Resources

Principal Lead Investigator(s):

Lehman, Peggy

Poulton, Nicole

Mueller-Solger, Anke

Amount Requested: \$248,289

TSP Panel Summary of Findings:

The external reviews were unanimous on the superior talent involved! One overall rating was superior. The power of spatial resolution in longitudinal transects from seawater to freshwater habitats supports the high technical cost and identification challenges this pilot study entails. The Pelagic Organism Decline is crucial to the endangered species, stocks and runs and I prefer this brave approach over a more cautious parsing of each problematic area. Huge space and time density study requires the flowcam to identify phyto and zooplankton to be coupled with direct sample counts. Some patterns and processes will require cautious sample analysis.

Questions/points to consider: How well does FlowCAM work, i.e., will it accomplish what is proposed?; To what degree will zooplankton be considered "identified"?; To what extent has instrument been used?; Is FlowCAM use valid for temporal and spatial sampling?; If FlowCam does work, what will the research provide in terms of furthering our understanding of Delta and plankton?; How well does the proposal address the topics set out in PSP? The project seems to focus on developing a tool that will address topics better, but it doesn't directly address hypotheses to be tested.

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TSP panel agrees that there will be low identification of zooplankton species. In terms of phytoplankton, it is believed that the FlowCAM will do a sufficient job. Researchers are trying to detect "patches" of phytoplankton. The identification of phytoplankton should reveal the temporal and spatial patterns. The Zooplankton identification should be sufficient to identify them as a food source.

Another concern brought forth by a panel member is that the FlowCAM is new technology and therefore a quarter of a million dollars may almost exclusively go into learning how the FlowCAM works. One external reviewer doesn't believe it would work on zooplankton and the panel is concerned about this.

There is an assumption in the proposal that the FlowCAM will do everything proposed. However, if the instrument doesn't work in the way proposed, the hypotheses may not be tested. If the instrument CAN do what is proposed, then the panel member would elevate the ranking. The Panel felt this proposal bordered on above average to superior, if conducted as a proof-of-concept effort.

Relevance to PSP Topic Areas:

High

TSP Technical Rating:

Above Average

TSP Funding Recommendation:

Fund w/conditions

TSP Amount Recommended: \$150,000

Conditions:

The panel was very interested in the technology, recognizing it as a potential opportunity to make a major contribution. However, the panel was concerned about the efficiency of the FlowCAM. Therefore, the panel requests to change the project to a "Proof-of-Concept" project.

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1. The panel requests that all Year-1 sampling be retained to validate efficacy across the range of conditions and drop all Year-2 sampling.
2. The panel recommends reducing the labor for tasks 2 and 3 by \$40,000.
3. The panel members suggest that the applicant lease the equipment vs. purchasing it. The panel assumes the purchase price is \$81,000 and the lease price is \$21,000, resulting in a reduction of \$60,000.

External Technical Review #1

Proposal Title: Using FlowCAM technology to measure high frequency spatial and temporal variation in phytoplankton and zooplankton species composition and develop state-of-the-art plankton monitoring programs

Proposal Number: 0056

Proposal Applicant: California Department of Water Resources

Purpose

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| Comments | <p>This project proposes to develop improved methodology for quantifying phytoplankton and zooplankton biomass and species composition centered on the recently developed instrument called the "FlowCAM" that pumps water samples through a small observation chamber and takes digital pictures of each object and measures chlorophyll fluorescence. Based on these digital images, size measurements and volume estimates can be computed, and these used to estimate biomass. Images can also be compared to stored images of known species, and a computer program can measure how similar the pictures are through a pixel to pixel comparison. This can be used to tag images that might be that particular species. In the end, however, species can only be identified based on that single image, and it still takes a knowledgeable expert to examine each image individually to identify the image to classify it on some taxonomic level. This proposal suggests that by finding a more efficient, lower cost method for characterizing plankton populations a better understanding of the relationships between fish recruitment and plankton populations can be achieved. This project focuses mainly on phytoplankton populations based on the premise that these regulate zooplankton populations, and zooplankton are the primary food of larval and juvenile fishes.</p> <p>Compared to other areas of marine/aquatic science,</p> |
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| | <p>very few scientists quantify phytoplankton populations to species due to the tedious, time consuming labor involved. Enumeration of zooplankton samples to species is done more commonly. For the past several decades there have to numerous attempts to develop instruments that will automate at least part of this process. Each of them has its strengths and weaknesses. The FlowCAM produces an archival record of the images of all objects within a particular size range. It can distinguish chlorophyll containing objects from other objects based on the fluorescence signature of chlorophyll a. This assists with separating phytoplankton from zooplankton, but it is not completely accurate since many heterotrophic protozoa may contain food vacuoles with phytoplankton, or have assimilated chloroplasts from their prey (kleptochloroplasty).</p> |
| Rating | Sufficient |

Background

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| Comments | <p>I think the premise of this proposal is sound. However it is clear that the PIs have never worked with this system (FlowCAM). They seem to completely buy the sales pitch of the folks trying to sell the instrument (Poulton, Nelson) and have not spoken with scientists with first-hand experience with this system. They propose to characterize phytoplankton over a wide range of sizes by changing the microscope objectives on this system. Good enough, but if you change magnifications too much, you also have to change the flow chamber, which are optimized to work with cells within certain size ranges. This then requires several runs with each sample and extensive changing and adjusting of the system in the field. Furthermore, no one has much experience with using these for enumerating preserved zooplankton samples. Clearly this uses much lower magnification and a much larger flow chamber. One needs to keep the samples suspended somehow while pumping it through, and avoid having the organisms clump together. Yes, it is possible to get</p> |
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| | <p>very nice pictures as are shown in Figure 2, but the practical realities of using this system involve lots of issues with clogs, clumped organisms etc. It is also apparent from the images of zooplankton in Figure 2 that the system, which was designed for smaller objects, does not work as well for larger object. Notice the bright "flares" in the images of copepods. Additional work will be needed to optimize the optics for zooplankton, to work out methods to keep the samples from clumping to get images of single individuals etc. Furthermore, the image recognition software is completely untested on organisms with complex shapes and movable body parts like copepods. For example, the antennae may be extended at right angles, folded by the side, bent etc. Will the computer be able to recognize these as the same species? I think not.</p> |
| Rating | Sufficient |

Approach

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| Comments | <p>I think the proposed field sampling proposed is sound, and the goals of the project are simple and straight-forward. Again, I thin the biggest issues with the approach is a lack of appreciation by the PIs for the limitations and technical issues associated with the use of the FlowCAM. Either they have not read, or they choose to gloss over the findings of the few published studies where the FlowCAM has been used to characterize plankton communities. While these PI's propose to use the instrument to automatically identify all the species in their samples, in the study of See et al., 2005, which they cite as an example of this instrument enumerating plankton samples, phytoplankton were characterized simply as "diatoms" or "other nanoplankton", and this was not done using the automated image recognition, but by an person looking at each</p> |
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| | image and classifying it simply as a diatom or other phytoplankton. |
| Rating | Sufficient |

Feasibility

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| Comments | I think the PIs will find it extraordinarily challenging to try and use the image recognition software to classify each individual in their samples to genus or species. Normally, you do this a single species at a time. You use multiple images of each species, since you don't know in advance the orientation or size of individuals from you sample as they pass through the observation chamber and are imaged. If you set your recognition threshold too high, you will miss many of the target organisms; if you set it too low, you will correctly tag most of the target organisms but you will misidentify a large number of other objects as your target category. Each image needs to be examined by a trained person to confirm or reject the identification made by the computer. The real strength of the FlowCAM is in rapidly producing a set of photographs of all the organisms in the sample, and making measurements on them (Size, volume). Someone still needs to look at each image and try to identify it based on that single image. To think that the computer matching software can do this reliably is not realistic. |
| Rating | Inadequate |

Budget

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| Comments | I think the budget seemed clear and reasonable, although I didn't spend much time scrutinizing it due to the other weaknesses I found in the overall proposal. |
| Rating | Above Average |

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External Technical Review #1

Relevance To CALFED

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| Comments | I think this study fits well into the priorities stated in the PSP. I think only a pilot or demonstration project is justified at this point, so it can be determined if this technology has any promise in this particular application. |
| Rating | Sufficient |

Qualifications

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| Comments | The PI's has an excellent publication and research record. However, she has no experience using this instrument and is relying on the claims of the people trying to sell her this instrument. |
| Rating | Superior |

Overall Evaluation Summary Rating

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| Comments | I can not recommend this full project as high priority for funding. I think a pilot study might be justified. Perhaps the PI could rent a system for a few months, become familiar with it, and then have a better understanding of its strengths and weaknesses. Then a more realistic full proposal could be written. |
| Rating | Sufficient |

External Technical Review #2

Proposal Title: Using FlowCAM technology to measure high frequency spatial and temporal variation in phytoplankton and zooplankton species composition and develop state-of-the-art plankton monitoring programs

Proposal Number: 0056

Proposal Applicant: California Department of Water Resources

Purpose

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| Comments | - The goals, objectives, and hypotheses are clearly stated with objectives tied to testable hypotheses. The proposal is justified as addressing mainly Topic 4 of the CALFED Priority Research Topic List. The topic of phytoplankton and zooplankton community structure is at the heart of ecosystem function and as such is of great interest to CALFED restoration efforts. - The results of this study will add to the base of knowledge by providing simultaneous measurements of plankton community structure. |
| Rating | Above Average |

Background

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| Comments | - A conceptual model is proposed but only mentions that the mismatch of phytoplankton to zooplankton is an important factor in ecosystem function. - There is no a priori discussion as to what the authors think are the controlling factors or how they will serve to regulate community structure. - Yes, more measurements generally give us more information, but more information does not necessarily lead to better understanding. That is where a heuristic conceptual model would be most useful. |
| Rating | Sufficient |

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Approach

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| Comments | - The scientific approach is efficiently designed and organized into specific tasks. - Study directly addresses the objectives of the proposal. - The use of the FlowCAM may prove to be a valuable new monitoring method. I only wish that this technology had been put into a larger framework of predictive modeling. |
| Rating | Sufficient |

Feasibility

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| Comments | - Given the research history and experience of the PIs, I believe the feasibility of performing the work is very high. - The specific tasks are well discussed and probabilities of success well documented by previous data collection. - Prior collaboration of the PIs enhances potential for success. |
| Rating | Above Average |

Budget

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| Comments | - I question whether CALFED should be buying the central instrument for the project at \$81,230. - I seriously doubt that project should be matched by equipment (FluoroProbe) which was bought on previous CALFED grant. In addition, \$4,000 match for phytoplankton species identification also comes from previous CALFED grant. |
| Rating | Sufficient |

Relevance To CALFED

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| Comments | - CALFED priorities clearly state that projects should be interdisciplinary, synthesize existing information, or provide models of system level responses and connection. This study is interdisciplinary but does not address the other priorities. - The proposed statistical analysis of data at the end of the |
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External Technical Review #2

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| | experiment is not sufficient to rate as a predictive model. |
| Rating | Above Average |

Qualifications

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| Comments | - The PIs are highly qualified in this field of research and has vast experience in phytoplankton ecology. - PIs have strong backgrounds in regional issues. |
| Rating | Superior |

Overall Evaluation Summary Rating

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| Comments | There is potential for a wealth of new information to be produced by this study. All of this information will be most useful after it is integrated into future modeling efforts. However, the lack of a strong conceptual model and the disconnect between information and understanding through modeling greatly detracts from the proposal. |
| Rating | Sufficient |

External Technical Review #3

Proposal Title: Using FlowCAM technology to measure high frequency spatial and temporal variation in phytoplankton and zooplankton species composition and develop state-of-the-art plankton monitoring programs

Proposal Number: 0056

Proposal Applicant: California Department of Water Resources

Purpose

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| Comments | This is a pilot study to determine proof-of-concept for the use of new plankton identification and enumeration technology. The idea is timely. If the proof-of-concept works, there is great potential for future use in many pelagic environments beyond San Francisco Bay. The objectives, goals and hypotheses are stated explicitly in the proposal and are internally consistent. |
| Rating | Superior |

Background

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| Comments | The conceptual model is stated clearly and explains the underlying basis for the proposed work. However, the model presented does not consider the microbial loop or microzooplankton explicitly--these are major drivers of pelagic food webs. Some (but not all) of these organisms can be resolved by the proposed technology. In particular, microzooplankton are inevitably dietary components of the suspension-feeding mesozooplankton that are consumed by fish in the target ecosystem. For this reason, I rate this section of the proposal above average rather than superior. |
| Rating | Above Average |

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Approach

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| Comments | Overall the approach is well designed and is likely to meet the stated objectives of the project. There is a clear Management Plan, with appropriate resources in the budget. The anticipated products will be valuable, regardless of the outcome (i.e., yes, the sampling strategy works, vs. no, it does not). Longer term data management is not addressed in the proposal, but it is not necessary for this initial stage of the research. |
| Rating | Superior |

Feasibility

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| Comments | <p>Overall, the approach is feasible. However, the instrument that is the centerpiece of the proposed sampling, the FlowCAM, has not been used in any peer-reviewed studies of mesozooplankton (>200um). It has been used in several published studies of phytoplankton. The manufacturer's web site states that organisms as large as 3 mm can be identified and enumerated, but I have not located any published evidence that the instrument has been used for plankton larger than very small copepods. The two reports by Fluid Imaging Technologies cited in the proposal show that small mesozooplankton can be identified to fairly coarse taxonomic levels (not to species) and that their individual and cumulative biovolumes can be measured. No data are provided on actual enumeration at the species, or higher, level. I have some concern that the size of the flow chambers used in the Fluid Imaging reports (300 um and 800 um) may have limited the size of zooplankton that were visualized. I was unable to locate any information about the size range of particles/plankton sampled quantitatively by the individual flow cells.</p> <p>The gold standard here would be to do a thorough quantitative comparison of the same zooplankton samples analyzed using the FlowCAM and those analyzed</p> |
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External Technical Review #3

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| | by traditional microscopy with respect to numerical abundance, species composition, and biovolume. While this base is covered to some extent in Task 5 of the proposal, in my opinion, it should be front and center as a primary objective of the research. If the FlowCAM is adequate for identification and enumeration of live and/or preserved mesozooplankton, this is a valuable result. If not, the result is still valuable. |
| Rating | Sufficient |

Budget

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| Comments | Each proposed task and associated expenses are specified in the budget. The budget is reasonable for the work proposed. The cost-sharing is a plus. |
| Rating | Superior |

Relevance To CALFED

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| Comments | The proposed work is highly relevant to CALFED's priorities and addresses more than one of the topics in the PSP. If improved and efficient plankton sampling for monitoring purposes results from the work, this will ultimately be highly valuable to resource managers as well as to scientists and modelers studying pelagic ecosystems. |
| Rating | Superior |

Qualifications

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| Comments | The primary investigators all have excellent records of research performance and publication. Appropriate infrastructure is available to support the project. |
| Rating | Superior |

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External Technical Review #3

Overall Evaluation Summary Rating

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| Comments | This is a good idea that needs to be evaluated quantitatively. It is highly responsive to Calfed's needs, particularly if it results in efficient, cost-effective monitoring in the future. |
| Rating | Superior |

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